Genomic integration of the full-length dystrophin coding sequence in Duchenne muscular dystrophy induced pluripotent stem cells.

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Public Summary:
This study focused on genetic engineering strategies to address Duchenne muscular dystrophy. The gene mutated in this disorder is very large. Therefore, special methods were needed to assemble and express the full-length DMD gene in bacteria. Plasmids that carried the entire gene DMD gene were developed that also include useful marker genes like luciferase and mCherry, for tracking plasmid expression in cells and tissues. The plasmid carry two intron sequences in the DMD gene that improve the yield of plasmid DNA in bacteria. The plasmid also carries a DNA sequence that allows the vector to become integrated into the genome of human cells in the presence of a site-specific recombinase enzyme called phiC31 integrase. In this work, we demonstrated the incorporation of the DMD plasmid into human chromosomes in human tissue culture cells and expression of the DMD gene. In addition, we demonstrated incorporation of the DMD plasmid into induced pluripotent stem cells derived from a patient with DMD. The iPS cells previously did not express any DMD, but after incorporation of our plasmid, they expressed the correct DMD protein. Furthermore, the plasmid was used in gene therapy experiments to introduce the DMD gene into the hind limb muscles of a mouse model of DMD. The plasmid was introduced by a vascular muscle infusion method and resulted in muscle fibers that now expressed DMD, whereas no DMD expression is normally present in these mice. Therefore, the plasmids we developed have utility in cell and gene therapy strategies to treat Duchenne muscular dystrophy.

Scientific Abstract:
The plasmid vectors that express the full-length human dystrophin coding sequence in human cells was developed. Dystrophin, the protein mutated in Duchenne muscular dystrophy, is extraordinarily large, providing challenges for cloning and plasmid production in Escherichia coli. The authors expressed dystrophin from the strong, widely expressed CAG promoter, along with co-transcribed luciferase and mCherry marker genes useful for tracking plasmid expression. Introns were added at the 3' and 5' ends of the dystrophin sequence to prevent translation in E. coli, resulting in improved plasmid yield. Stability and yield were further improved by employing a lower-copy number plasmid origin of replication. The dystrophin plasmids also carried an attB site recognized by phage phiC31 integrase, enabling the plasmids to be integrated into the human genome at preferred locations by phiC31 integrase. The authors demonstrated single-copy integration of plasmid DNA into the genome and production of human dystrophin in the human 293 cell line, as well as in induced pluripotent stem cells derived from a patient with Duchenne muscular dystrophy. Plasmid-mediated dystrophin expression was also demonstrated in mouse muscle. The dystrophin expression plasmids described here will be useful in cell and gene therapy studies aimed at ameliorating Duchenne muscular dystrophy.